IHC Double staining kit(Polymer-HRP)(DAB/AEC, For Mouse/Rabbit Primary Antibodies)

Catalog No.: PA10002

Basic Information

Product name	IHC Double staining kit(Polymer-HRP)(DAB/AEC, For Mouse/Rabbit Primary Antibodies)
Sizes	6 mL, 30 mL, 100 mL
Storage	2-8℃
Shipping	Shipped with ice pack
Validity	12 months

Product Introduction

EnkiLife's dual immunohistochemistry detection system enables simultaneous detection of two distinct antigens on the same tissue section. The Polymer-HRP-based IHC detection system employs advanced polymer technology to conjugate horseradish peroxidase (HRP) with specific antibodies, forming a stable polymer complex. This unique labeling approach significantly enhances signal intensity and detection sensitivity while minimizing background staining, offering user-friendly operation and robust stability. The kit includes Polymer-HRP-labeled anti-mouse and anti-rabbit secondary IgGs, paired with DAB (brown) and AEC (red) chromogenic substrates, to achieve dual-antigen localization and visualization.

Product Components

Components	6 mL	30 mL	100 mL
Reagent 1: 3 % H_2O_2	6 mL	30 mL	100 mL
Reagent 2: Goat serum blocking solution	12 mL	60 mL	200 mL
Reagent 3: Polymer-HRP-conjugated goat anti-mouse IgG	6 mL	30 mL	100 mL
Reagent 4: Polymer-HRP-conjugated goat anti-rabbit IgG	6 mL	30 mL	100 mL
Reagent 5: DAB concentrate (50×)	120 µL	600 µL	2 mL
Reagent 6: DAB diluent	6 mL	30 mL	100 mL
Reagent 7: AEC chromogen A	300 µL	1.5 mL	5 mL

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Reagent 8: AEC chromogen B	300 µL	1.5 mL	5 mL
Reagent 9: AEC chromogen C	6 mL	30 mL	100 mL

Experimental procedure

1. Deparaffinization and rehydration: Dewax formalin-fixed paraffin-embedded (FFPE) sections in xylene and rehydrate through graded ethanol.
2. Antigen retrieval: Perform heat-induced epitope retrieval (HIER) or enzymatic retrieval as required by the primary antibodies.
3. Endogenous peroxidase quenching: Incubate slides with 3 % H_2O_2 (Reagent 1) for 15–20 min at RT.
4. Wash: 3 × 3–5 min with PBST.
5. Blocking: Cover tissue with goat serum blocking solution (Reagent 2) and incubate 30 min at 37 °C.
6. Primary antibody incubation: After determining the optimal dilution, evenly overlay the tissue section with the working antibody solution and incubate overnight at 4 °C or for 1 h at 37 °C.
7. Wash: 3 × 3–5 min PBST.
8. Secondary antibody incubation: Add Polymer-HRP-labeled goat anti-mouse IgG (Reagent 3) to the section [select the appropriate secondary antibody according to the primary antibody species] and incubate at room temperature for 30-60 minutes.
9. Wash: 3 × 3–5 min with PBST.
10. Chromogenic reaction: Apply DAB substrate (diluted 1:50, e.g., 1 µL DAB concentrate—briefly centrifuge Reagent 5 before use—mixed with 49 µL DAB diluent, Reagent 6) onto the tissue section. Prepare the working solution immediately before use and consume within 30 min. Monitor color development under a light microscope and terminate the reaction promptly once optimal signal intensity is achieved.
11. Wash: 3 × 3–5 min PBST.
12. Blocking: Repeat blocking step with goat serum (Reagent 2) for 30 min at 37 °C to saturate free binding sites.
13. Primary antibody incubation: After determining the optimal dilution, evenly overlay the tissue section with the working antibody solution and incubate overnight at 4 °C or for 1 h at 37 °C.
14. Wash: 3 × 3–5 min PBST.
15. Secondary antibody incubation: Add Polymer-HRP-labeled goat anti-rabbit IgG (Reagent 4) to the section [select the appropriate secondary antibody according to the primary antibody species] and incubate at room temperature for 30-60 minutes.
16. Wash: 3 × 3–5 min PBST.

17. Chromogenic reaction: Apply AEC substrate (prepared fresh at 1:20; e.g., centrifuge Reagents 7 and 8 briefly, then combine 10 µL AEC chromogen A, 10 µL AEC chromogen B, and 180 µL AEC chromogen C) onto the tissue section. Use the working solution within 30 min of preparation. Monitor color development under a light microscope and terminate the reaction promptly once the desired signal intensity is reached.
18. Counterstain: Stain nuclei with Mayer' s hematoxylin (aqueous formulation; avoid alcohol-containing solutions).
19. Mounting: Use an aqueous mounting medium (e.g., glycerol gelatin). Do not dehydrate through ethanol or clear in xylene.
20. Microscopy: Visualize dual-color staining by bright-field microscopy.

Notes

1.Store all reagents at 2–8 °C; do not freeze.
2.Optimize primary antibody dilutions and incubation times empirically.
3.AEC precipitate is alcohol-soluble; avoid alcoholic counterstains, differentiation solutions, ethanol dehydration, and xylene clearing. Use only aqueous mountants.
4.Protect AEC substrate and final slides from light to prevent chromogen fading.
5.Wear appropriate personal protective equipment (lab coat, gloves, mask).
6.For research use only; not intended for diagnostic or therapeutic applications.

This product is for research use only!